Ing2 Cas9-CKO Strategy

Designer: Yanhua Shen

Reviewer: Xueting Zhang

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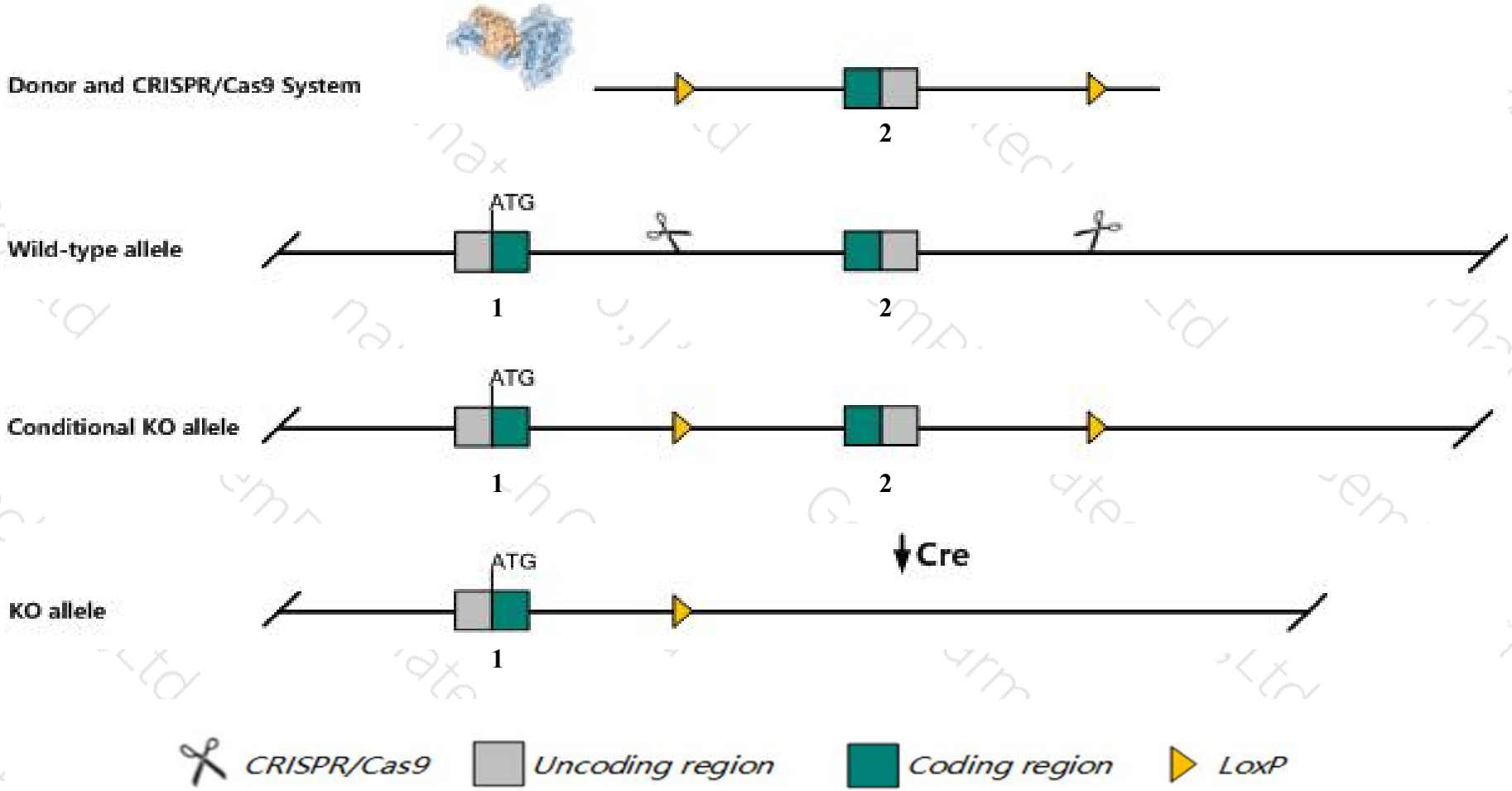
Project Overview

Project Name	***Ing2***
Project type	**Cas9-CKO**
Strain background	**C57BL/6JGpt**

Conditional Knockout strategy

This model will use CRISPR/Cas9 technology to edit the *Ing2* gene. The schematic diagram is as follows:



Technical routes

- The *Ing2* gene has 3 transcripts. According to the structure of *Ing2* gene, exon2 of *Ing2-201* (ENSMUST00000080353.2) transcript is recommended as the knockout region.The region contains most of coding sequence. Knock out the region will result in disruption of protein function.

- In this project we use CRISPR/Cas9 technology to modify *Ing2* gene. The brief process is as follows:CRISPR/Cas9 system and Donor were microinjected into the fertilized eggs of C57BL/6JGpt mice.Fertilized eggs were transplanted to obtain positive F0 mice which were confirmed by PCR and sequencing. A stable F1 generation mouse model was obtained by mating positive F0 generation mice with C57BL/6JGpt mice.

- The flox mice will be knocked out after mating with mice expressing Cre recombinase, resulting in the loss of function of the target gene in specific tissues and cell types.

Notice

- According to the existing MGI data,homozygous inactivation of this gene causes impaired spermatogenesis and male infertility associated with teratozoospermia, seminiferous tubule degeneration, germ cell depletion, arrest of male meiosis and enhanced testicular apoptosis, and leads to an increased incidence of soft tissue sarcomas.
- The *Ing2* gene is located on the Chr8. If the knockout mice are crossed with other mice strains to obtain double gene positive homozygous mouse offspring, please avoid the two genes on the same chromosome.
- This strategy is designed based on genetic information in existing databases.Due to the complexity of biological processes, all risk of loxp insertion on gene transcription, RNA splicing and protein translation cannot be predicted at existing technological level.

Gene information （NCBI）

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Ing2 inhibitor of growth family, member 2 [Mus musculus (house mouse)]

Gene ID: 69260, updated on 13-Mar-2020

Summary

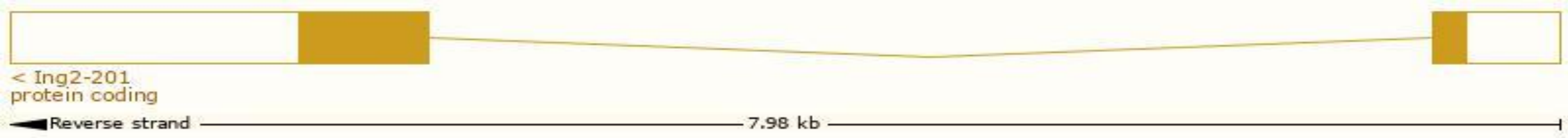
Official Symbol	Ing2 provided by MGI
Official Full Name	inhibitor of growth family, member 2 provided by MGI
Primary source	MGI:MGI:1916510
See related	Ensembl:ENSMUSG00000063049
Gene type	protein coding
RefSeq status	VALIDATED
Organism	Mus musculus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Myomorpha; Muroidea; Muridae; Murinae; Mus; Mus
Also known as	2810011M06Rik, Ing1l, Ing2b, P33ING2
Expression	Ubiquitous expression in placenta adult (RPKM 8.8), cortex adult (RPKM 5.3) and 28 other tissues See more
Orthologs	human all

Transcript information（Ensembl）

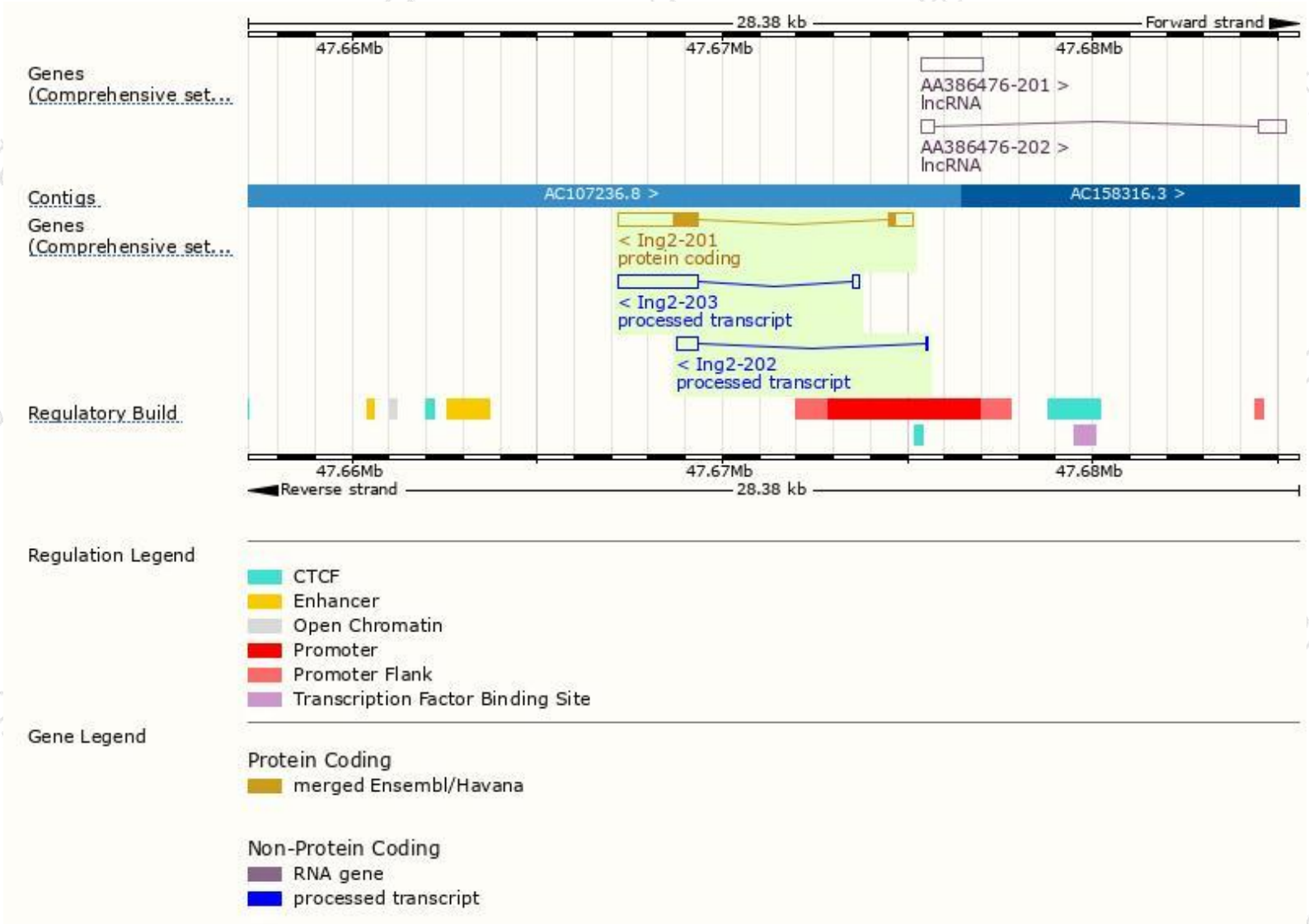
The gene has 3 transcripts,all transcripts are shown below:

Name	Transcript ID	bp	Protein	Biotype	CCDS	UniProt	Flags
Ing2-201	ENSMUST00000080353.2	2812	281aa	Protein coding	CCDS22298	Q9ESK4	TSL:1 GENCODE basic APPRIS is a system to annotate alternatively spliced transcripts based on a range of computational methods to identify the most functionally important transcript(s) of a gene. APPRIS P1
Ing2-203	ENSMUST00000146625.2	2320	No protein	Processed transcript	-	-	TSL:1
Ing2-202	ENSMUST00000125536.1	628	No protein	Processed transcript	-	-	TSL:3

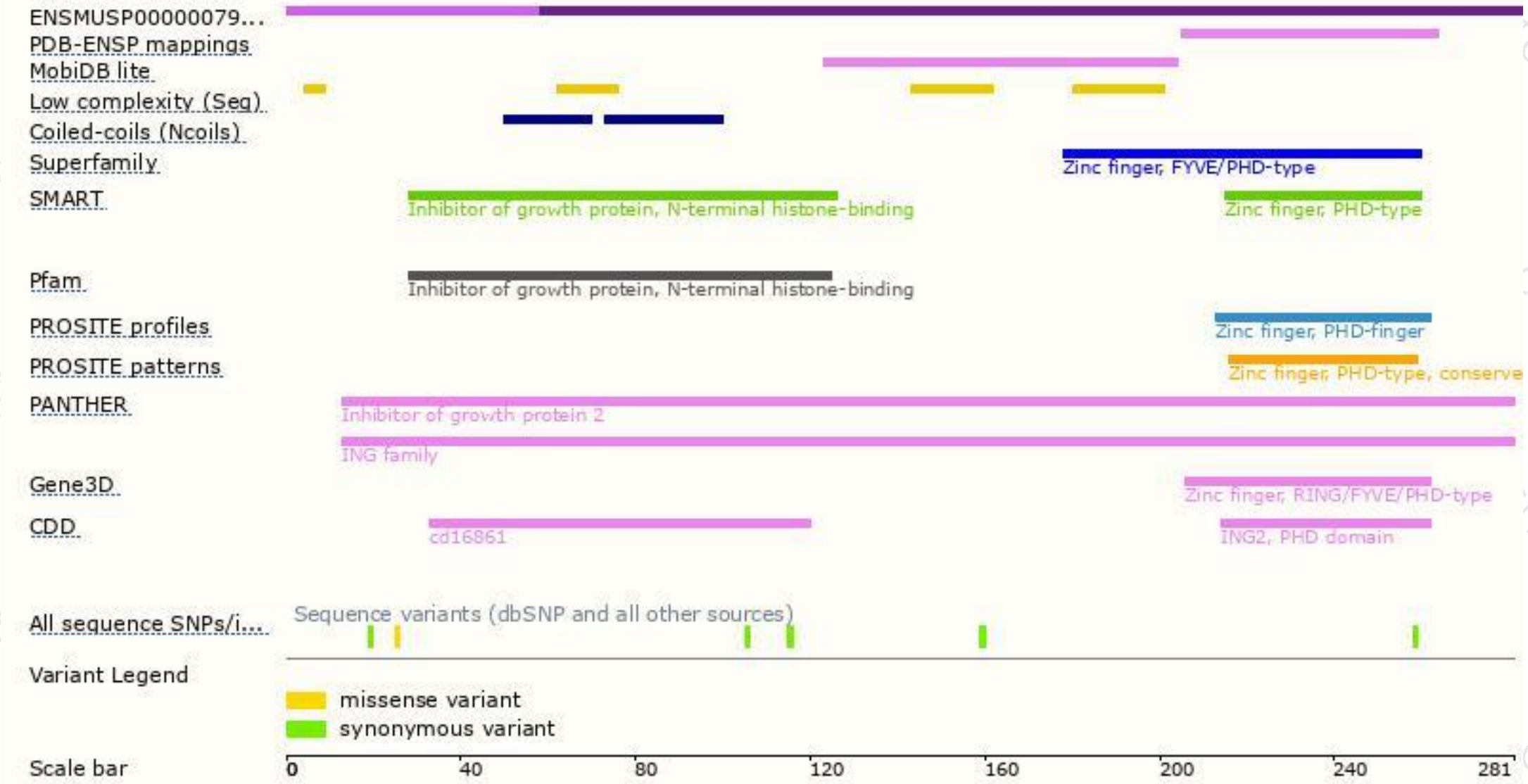
The strategy is based on the design of *Ing2-201* transcript,the transcription is shown below:



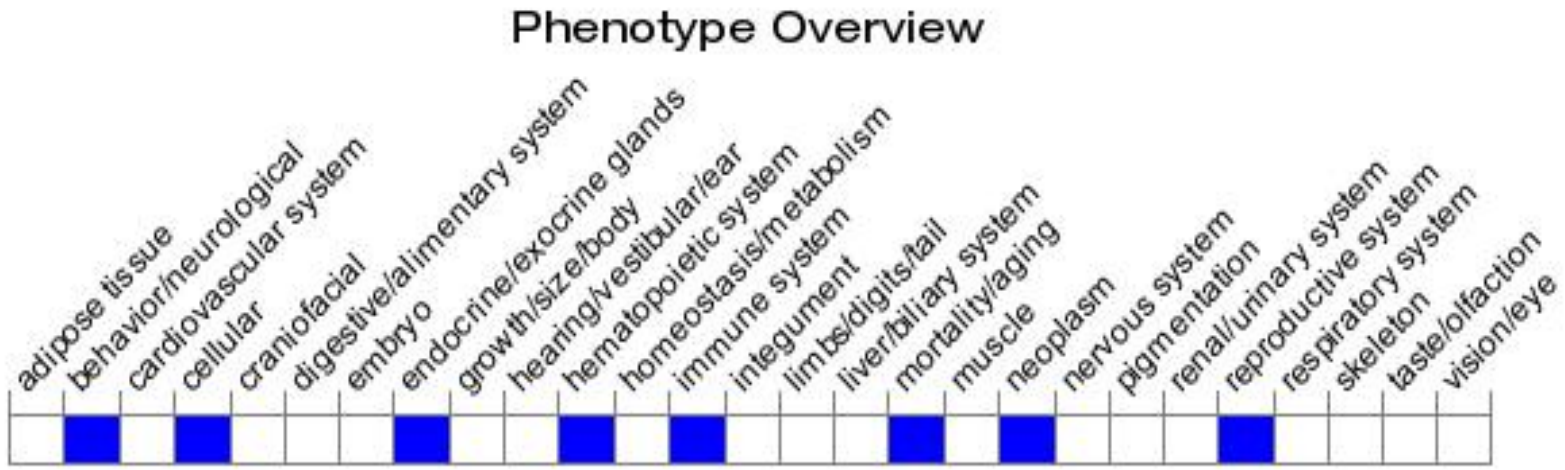
Genomic location distribution



Protein domain



Mouse phenotype description(MGI)



Phenotypes affected by the gene are marked in blue.Data quoted from MGI database(http://www.informatics.jax.org/).

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GemPharmatech

If you have any questions, you are welcome to inquire.

Tel: 400-9660890

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GemPharmatech Co.,Ltd